ANTIOXIDANT AND ANTIHYPERGLYCEMIC ACTIVITY OF *SOPHORA ALOPECUROIDES* SEED ON STREPTOZOTOCIN-NICOTINAMIDE INDUCED DIABETIC RATS

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DISSERTATION SUBMITTED IN FULFILMENT OF THE REQUIRMENTS FOR THE DEGREE OF MASTER OF BIOTECHNOLOGY

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Name of Degree: Master of Biotechnology

Title of Dissertation: Antioxidant and antihyperglycemic activity of *Sophora alopecuroides* seed on streptozotocin-nicotinamide induced diabetic rats

Field of Study: Biotechnology

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ABSTRACT

Sophora alopecuroides seed (SAS) was extracted with chloroform, ethanol and distilled water. The TLC result showed the presence of alkaloids in all of the extracts and the total alkaloid contents was 7.56%. The alkaloids were separated and identified with Q-TQF MS with known reference standard and were found that *Sophora alopecuroides* seed contained alkaloids namely sophocarpine, matrine, baptifoline, oxysophocarpine, oxymatrine, sophocarpine dimer, oxysophocarpine dimer, oxymatrine dimer and sophoranol-N-oxide dimer. The *in vitro* bioassays were performed to determine the antioxidant activity and glycogen phosphorylase enzyme inhibition activity of *Sophora alopecuroides* seed in ethanol and water extracts. In all of the bioassays, ethanol extract had showed highest activities. In DPPH assay the IC_{50} value of ethanol extract was 155.33 ± 0.06 µg/ml while in FRAP assay the IC_{50} value of ethanol extract was 9.71 ± 0.02 µg/ml. In GPa enzyme assay the IC_{50} of ethanol *Sophora alopecuroides* seed extract was 581.61 µg/ml. Acute toxicity of ethanol *Sophora alopecuroides* seed extract was tested at increasing dose level in non-diabetic rats and no toxic effects were observed in male rats at a dose of 5 g/kg body weight. The ethanol *Sophora alopecuroides* seed extract at the dose of 500 mg/kg was capable of decreasing the glycemia of non-diabetic rats during an oral glucose tolerance test (OGTT). The treatment with SAS at the dose of 500 mg/kg to the diabetic rats for 28 days decreased fasting blood glucose levels significantly compared to the 0^{th} day. The 95% ethanol SAS extract at the dose of 250 mg/kg and 500 mg/kg body weight significantly ($^*P < 0.05$) decreased serum triglycerides and total cholesterol levels, and increased serum HDL levels compared to the diabetic control. Therefore, these results validate the traditional use of *Sophora alopecuroides* seed as an antidiabetic remedy.

ABSTRAK

Biji *Sophora alopecuroides* (SAS) telah diekstrakkan dengan klorofom, etanol dan air suling. Keputusan Keratan Lapisan Nipis (KLN) menunjukan kehadiran alkaloid didalam kesemua ekstrak and jumlah kandungan alkaloid adalah 7.56%. Alkaloid telah diasingkan dan dikenalpastikan menggunakan Q-TQF MS dengan rujukkan piawai dan didapati mengandungi alkaloid sophocarpine, matrine, baptifoline, oxysophocarpine, oxymatrine, sophocarpine dimer, oxysophocarpine dimer, oxymatrine dimer dan sophoranol-N-oxide dimer. Bioasei *in vitro* telah dijalankan untuk menentukan aktiviti antioksidan dan aktiviti perencatan enzim fosforilase ekstrak etanol dan air biji *Sophora alopecuroides*. Dalam kesemua bioasei, ekstrak etanol menunjukan aktivit tertinggi. Dalam asei DPPH nilai IC_{50} ekstrak etanol adalah 155.33 ± 0.06 ug/ml manakala dalam asei FRAP nilai IC_{50} ekstrak etanol adalah 9.71 ± 0.02 ug/ml. Didalam asei enzim GPa IC_{50} ekstrak etanol biji *Sophora alopecuroides* adalah 581.61 ug/ml. Ketoksikan akut ekstrak etanol biji *Sophora alopecuroides* telah diujikan pada paras dos menaik dalam tikus tak-dibetik dan tiada kesan ketoksikan dilihat di tikus jantan pada dos 5 g/kg berat badan. Ekstrak etanol biji *Sophora alopecuroides* pada dos 500 mg/kg berkebolehan menurunkan glisemia tikus tak-dibetik semasa ujian oral ketahanan glukosa (OGTT). Perlakuan dengan SAS pada dos 500mg/kg tikus diabetic signifikan merendahkan paras glukosa berbanding pada hari 0. Ekstrak 95% etanol SAS pada dos 250 mg/kg dan 500 mg/kg berat badan menurun signifikan (*$P<0.05$) dengan serum trigliserida dan jumlah paras kolesterol dan meningkatkan paras serum HDL berbanding kawalan diabetik. Oleh itu, keputusan ini mengesahkan penggunaan traditional biji *Sophora alopecuroides* sebagai remedi antidiabetik.

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ATP	adenosine tree phosphate
$CHCl_3$	Chloroform
DMSO	dimethyl sulfoxide
DPPH	2, 2-diphenyl-1-picrylhydrazyl
DPP-IV	dipeptidyl peptidase-IV
EDTA	ethylene-diamine-tetraacetic acid
EGTA	ethylene glycol tetraacetic acid
ELISA	enzyme-linked immunosorbent assay
ESI	electrospray ionization
EtOH	ethanol
Ext	extract
FFA	free-fatty acid
FPG	Fasting plasma glucose
FRAP	ferric reducing antioxidant power
G1P	glucose 1-phosphate
G6P	glucose 6-phosphate
GDM	gestational diabetes mellitus
GIP	glucose-dependent insulinotropic polypeptide

GLP-1	glycogen like peptide-1
GP	glycogen phosphorylase
Gpi	glycogen phosphorylase inhibitor
H_2O	distilled water
H_2SO_4	sulphuric acid
HbA1c	glycosylated haemoglobin
HCI	hydrochloric acid
HDL	high density lipoprotein
HPLC	high performance liquid chromatography
IC_{50}	half maximal inhibitory activity
IGT	impaired glucose tolerance
KCI	potassium chloride
L	litter
ml	milliliter
µl	microliter
m	meter
cm	centimeter
Kg	kilogram
g	gram
mg	milligram

µg	microgram
M	molarity
mM	millimolar
µM	micromolar
mmol	millimole
min	minute
MW	molecular weight
MeOH	methanol
MgCI2	magnesium chloride
NaOH	sodium hydroxide
NF-kB	nuclear factor kappa B
NIDDM	non-insulin dependent diabetes mellitus
OGTT	oral glucose tolerance test
PKC	protein kinase C
PPAR-γ	peroxisome proliferation activated receptor-gamma
Q-TOF MS	quadruple time-of-flight mass spectrometer
Rf	relative mobility
RNS	reactive nitrogen species
ROS	reactive oxygen species
rpm	revolutions per minute

SAS	*Sophora alopecuroides* seed
SD	standard deviation
SEM	standard error of the mean
SGLT	sodium glucose co-transporter
STZ	streptozotocin
SUR	sulfonylurea receptor
TC	total cholesterol
TCA	trichloroacetic acid
TG	triglycerides
TLC	thin layer chromatography
TZD	thiazolidinediones
TNF-a	tumour necrosis factor-a
UV	ultraviolet
WKY	Wistar Kyoto rats
WHO	World Health Organization
^{o}C	degree Celsius
%	percentage
≤	Less than or equal to
≥	More than or equal to